JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Article

Impact of Three Thiazolidinone Compounds with Piperine Skeletons on Trehalase Activity and Development of *Spodoptera frugiperda* Larvae

Bin Tang, Shangrong Hu, Yujia Luo, Dongmei Shi, Xiangyu Liu, Fan Zhong, Xinyi Jiang, Gao Hu, Can Li, Hongxia Duan,* and Yan Wu*

Cite This: *J. Agric. Food Chem.* 2024, 72, 8423−8433

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Trehalases (TREs) are pivotal enzymes involved in insect development and reproduction, making them prime targets for pest control. We investigated the inhibitory effect of three thiazolidinones with piperine skeletons (6a, 7b, and 7e) on TRE activity and assessed their impact on the growth and development of the fall armyworm (FAW), *Spodoptera frugiperda*. The compounds were injected into FAW larvae, while the control group was treated with 2% DMSO solvent. All three compounds effectively inhibited TRE activity, resulting in a significant extension of the pupal development stage. Moreover, the treated larvae exhibited significantly decreased survival rates and a higher incidence of abnormal phenotypes related to growth and development compared to the control group. These results suggest that these TRE inhibitors affect the molting of larvae by regulating the chitin metabolism pathway, ultimately reducing their survival rates. Consequently, these compounds hold potential as environmentally friendly insecticides.

KEYWORDS: *trehalase inhibitor, Spodoptera frugiperda, growth and development, chitin metabolism, abnormal phenotype*

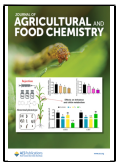
1. INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a native crop pest in the Americas and was first discovered in the subtropical and tropical regions of Africa. This insect is one of the most notorious pests worldwide.[1−3] *S. frugiperda* has two subtypes: corn (C-type) and rice (R-type).[4−7] Owing to its voracious, nurturing, adaptable, and drug-resistant characteristics, it can spread rapidly and has been classified as a major migratory agricultural pest.[8,9] Currently, the main chemical pesticides used for prevention include neonicotinoids, organophosphates, bisamides, and other pesticides, but the FAW has developed multi-insecticide resistance.[10,11] However, the reliance on chemical pesticides and the increase in pesticide usage and dosage have raised concerns regarding the safety of agricultural products, the environment, and biodiversity.[12] Additionally, it has led to the development of resistance to different insecticides in *S. frugiperda*,[10,13−15] which is detrimental to sustainable agricultural practices. Therefore, the development of environmentally friendly and sustainable pest control strategies is imperative.[16,17]

Trehalose is a nonreducing disaccharide composed of two glucose units linked via an α-α-1,1-glycosidic bond. It is present in various invertebrates such as fungi, bacteria, plants, nematodes, and insects.[18−20] Trehalase (TRE), a glucosidase, is the only enzyme that irreversibly hydrolyzes trehalose into two glucose molecules. There are two different forms of TRE in insects: soluble trehalase (TRE1) and membrane-bound trehalase (TRE2),[21−24] which work in concert to maintain the insect's energy supply. Chitin, a vital component enveloping the entire body of insects, encompassing the exoskeleton and gut, plays a pivotal role in safeguarding and reinforcing the extracellular matrix while enclosing the nutrient matrix.[25] TRE serves as the first enzyme in the chitin synthesis pathway,[26,27] establishing a link with the trehalose metabolism pathway. Consequently, the discovery and development of TRE inhibitors that regulate the chitin metabolism pathway have become increasingly important in pest control strategies.

TRE inhibitors possess a structure resembling that of trehalose. They can strongly bind with amino acids on the active site of TRE through hydrogen bonds to form complexes and competitively inhibit the activity of TRE.[28−30] This inhibition disrupts the decomposition and metabolism of trehalose in insects, ultimately leading to insect mortality. Several analogues targeting TRE have been proposed as inhibitors. These include Validamycin, Sudatrastin, Trehazolin, and their derivatives, which have been isolated from natural sources. Additionally, Validamycin A,[31,32] Validoxylamine A,[33,34] Trehazolin,[35,36] and Salbostatin,[37] have been purified by through synthetic methods. Furthermore, nonglycosyl

Received: November 27, 2023
Revised: March 14, 2024
Accepted: March 15, 2024
Published: April 2, 2024



https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

inhibitors such as piperine compounds can also serve as potential inhibitor frameworks for TRE.

Piperine derivatives such as ZK-PI-5 ($C_{20}H_{19}NO_4$) and ZK-PI-9 ($C_{19}H_{16}ClNO_3$) have emerged as TRE inhibitors that regulate insect development and reproduction.[38−41] Piperine compounds contain a unique skeleton of 1,3-benzodioxane and exhibit multiple biological activities. They possess a simple structure and are harmless to nontarget organisms.[42,43] Thiazolidone contains multiple reaction centers that can be modified by introduction into compounds generated on the piperine skeleton to inhibit Chitinase activity in insects.[42] These compounds have shown promise in preventing and controlling lepidopteran pests, such as cotton bollworms and armyworms, and have lethal effects on aphids, *Ostrinia furnacalis*, and other pests.[44,45] Moreover, they demonstrated the ability to inhibit TRE activity in *S. frugiperda* larvae.

In this study, we introduced a thiothiazolidone structural fragment into the original piperine skeleton to design and synthesize piperazole thiothiazolidone compounds 6a, 7b, and 7e. By investigating the effects of compounds 6a, 7b, and 7e on the growth and development of *S. frugiperda* larvae, we aimed to assess their potential as TRE inhibitors, offering novel insights into the development of environmentally friendly sustainable biopesticides.

2. MATERIALS AND METHODS

2.1. Insects. *S. frugiperda* used in this study was provided by the Zhejiang Academy of Agricultural Sciences (Zhejiang, Hangzhou) and was subsequently reared at the Key Laboratory of Animal Adaptation and Evolution, School of Life and Environmental Sciences, Hangzhou Normal University (Zhejiang, Hangzhou). Female adult moths were fed 10% honey water to lay eggs, which were collected for hatching in a feeding box with dimensions of 4.9 cm (top diameter), 3.6 cm (bottom diameter), and 4.2 cm (height) (Junyuan Experimental Equipment Store, Nantong, China). The newly hatched larvae were fed an artificial diet and transferred to individual feeding boxes with dimensions of 2.9 2 4, and 8.3 cm (height) once they reached the third instar. Adults and larvae were maintained in a controlled environment with a temperature of 26 ± 1 °C, relative humidity of 60 ± 10%, and a 16 h/8 h (day/night) photoperiod. Artificial feed formulation was improved according to the method proposed by Pinto et al., as shown in Table 1.[46]

Table 1. Ingredient of Artificial Feed

ingredient	amount
Sspotted bean powder	111.0 g
yeast powder	33.8 g
wheat germ	52.8 g
ascorbic acid	3.4 g
methyl p-hydroxybenzoate	2.1 mL
sorbic acid	1.1 g
10% formaldehyde	8.4 mL
water (stirring)	490.0 mL
agar	13.5 g
water (agar solution)	338.0 mL

2.2. Piperine Compounds. The potential TRE inhibitors 6a, 7b, and 7e were provided by the PMDD Laboratory of China Agricultural University (Beijing). These inhibitors were dissolved in 2% DMSO to achieve a concentration of 2×10^{-3} mmol/mL based on the minimum effective concentration of validamycin on *S. frugiperda*.[39] Detailed information regarding the molecular weights and formulas of the TRE inhibitors is presented in Table 2, and their structural formulas are shown in Figure 1.

Table 2. Identification Codes, Molecular Weight (MW), and Molecular Formulas of Trehalase Inhibitors

code	purity	solvent	MW	molecular formula
6a	98%	DMSO	341.399	$C_{17}H_{11}NO_3S_2$
7b	98%	DMSO	357.442	$C_{18}H_{15}NO_3S_2$
7e	98%	DMSO	377.857	$C_{17}H_{12}ClNO_3S_2$

2.3. Microinjection of Trehalase Inhibitors. The TRE inhibitors were injected using glass capillaries transformed into microinjection glass needles with a Sutter Instrument (USA). These glass needles were installed into the injection joint of the Eppendorf TransferMan 4r microinjection system and securely fixed onto the robotic arm. *S. frugiperda* larvae were paralyzed on ice on the first day of the third instar. They were then transferred to the loading platform of the microinjection system by using a pointed brush. The needle was inserted into the thinner area between the second and third pairs of thoracic feet, and 300 nL of the potential inhibitors were injected into the larvae. An equal volume of DMSO (2%) was injected into the control group. Treated larvae were reared in artificial climate boxes.

2.4. Determination of Trehalase Activities and Carbohydrate Content. Samples were collected 48 h after the injection of 6a, 7b, 7e, and 2% DMSO for subsequent analyses. Approximately 9−15 larvae from each group were collected, and three biological replicates were performed. The collected samples were mixed with 200 μL of phosphate-buffered saline (PBS; pH 7.0) and subjected to sonication for 30 min of sonication. After adding 800 μL of PBS, the samples were centrifuged at 1000 × *g* at 4 °C for 20 min. Approximately 350 μL of supernatant was collected and used to determine the trehalose concentration (Anthrone method), glycogen content (glucose (GO) assay kit, USA), and protein concentration (Beyotime BCA reagent kit, China).[39] Another 350 μL of supernatant was collected and ultracentrifuged for 60 min. The resulting supernatant (300 μL) was collected and used to determine the activity of soluble trehalose, glucose content, and protein concentration using the respective kits. The precipitate was mixed with 300 μL of PBS to determine the activity of membrane-bound trehalose, glucose content, and protein concentration following the respective reagent kit instructions.

2.5. Quantitative Real-time Polymerase Chain Reaction (qRT-PCR). Total RNA was extracted from larvae collected 48 h after injection using TRIzol reagent according to the manufacturer's instructions. Three to five larvae from each group were collected, and five biological replicates were performed. The concentration and quality of the extracted RNA were determined by using a NanoDrop™ 2000 spectrophotometer. The RNA integrity was verified through 1% agarose gel electrophoresis. The RNA samples were stored at −80 °C for further analysis. Subsequently, cDNA was synthesized using the PrimeScript RT reagent kit with gDNA Eraser (NARISHIGE, Japan). The procedure was performed on ice.

qRT-PCR primers were designed based on the gene sequences of the trehalose and chitin metabolism pathways of *S. frugiperda* available from the National Center of Biotechnology Information (NCBI; USA) using Primer 5 software (Table 3). The primers were synthesized by Hangzhou Shangya Biotechnology Corporation (Hangzhou, China) and the ribosomal protein L10 gene (*RPL10*, GenBank ID:OK319023.1) of *S. frugiperda* was used as the internal reference gene.[47] Each 10 μL reaction volume contained 5 μL of SYBR Green Premium Ex Taq (Takara, Japan), 3.2 μL of deionized water, 0.4 μL of both forward and reverse primers, and 1 μL of cDNA sample. The PCR reaction procedure consisted of the following steps: predenaturation at 95 °C for 30 s, denaturation at 95 °C for 5 s, extension at 60 °C for 20 s, and 40 cycles. The relative expression levels of the genes were analyzed using the $2^{-\triangle\triangle CT}$ method.[48]

2.6. Determination of the Chitin Content. Larvae were collected in Eppendorf (EP) tubes 48 h after treatment. Each experiment consisted of three biological replicates and three technical replicates. To each EP tube was added 500 μL of 6% KOH solution, and the samples were incubated at 80 °C for 90 min. After shaking for 5 min, the samples were centrifuged at 12,000 × *g* at 4 °C for 20 min.

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

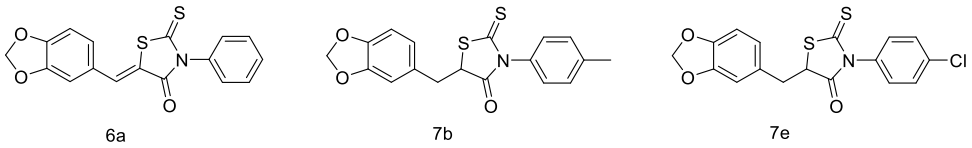


Figure 1. Molecular structures of three trehalase inhibitors: 6a, 7b, and 7e.

Table 3. Primers Sequences for qRT-PCR

gene name	forward primer (5′−3′)	reverse primer (5′−3′)	gene ID
TRE1	TCAGATGAAGGTGAACTCGAAGA	GGAATGATGAATCCGTGGGTA	DQ447188.1
TRE2	CTGCTGCTGTCGGAGATGA	TAGGAGGGGAGGCTGTGAT	EU872435.1
HK2	TTTCCACCCTCATTTCCACA	TTTGCTTAGGCGGCTTACAT	XM_035594594.1
G6PI	CACATGAAGGAGTTCTC	GCGTTGGTGATGGTCTCCTG	XM_035596038.1
GFAT	AGTCCTGTCAGTCATCCCTAT	AAATCCCAATCATTCGTTCA	XM_035573563.1
GNPNA	GAGTTGTTCGGAGACTGGTT	GGCTCTAATGGAACGCACTT	XM_035593197.1
PAGM	TCGATCAAGATTGACGTGAG	AGACCACATGTTCTAACAAGA	XM_035586036.1
UAP	ATGATGCTGCTAAGAAAGAC	CGTAAGATAGTAACGGTGAGA	XM_035601390.1
CHS2	TAGACTCTTGGGCGTTGACA	TTGGTTTCCTTCCCTTTCTC	AY525599.1
CHT	AAGCGGACAGCAAAGCG	CCAACTCAGGGTCAATAATAAGAAC	AY527414.1
RPL10	GACTTGGGTAAGAAGAAG	GATGACATGGAATGGATG	

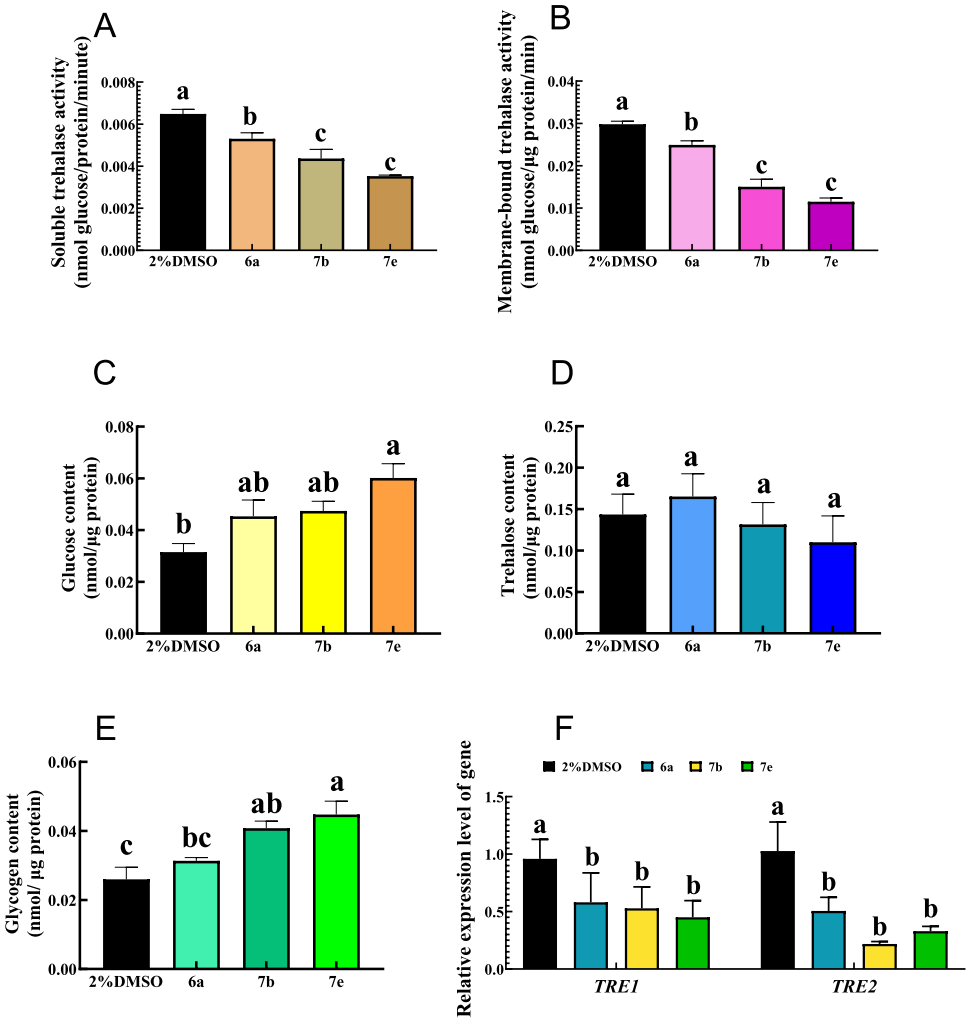


Figure 2. Effects of trehalase (TRE) inhibitors on TRE activity, trehalose, glucose, glycogen, and gene expression in *S. frugiperda* larvae 48 h after treatment. Changes in (A) soluble and (B) membrane-bound TRE activities. (C) Trehalose, (D) glucose, and (E) glycogen content. (F) TRE1 and *TRE2* gene expression. Values are presented as the means ± SE $*p < 0.05$ and $**p < 0.01$ denotes significant differences. ns: not significant (independent samples; *t* test); 2% DMSO: negative control group; 6a, 7b, and 7e: treatment groups.

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

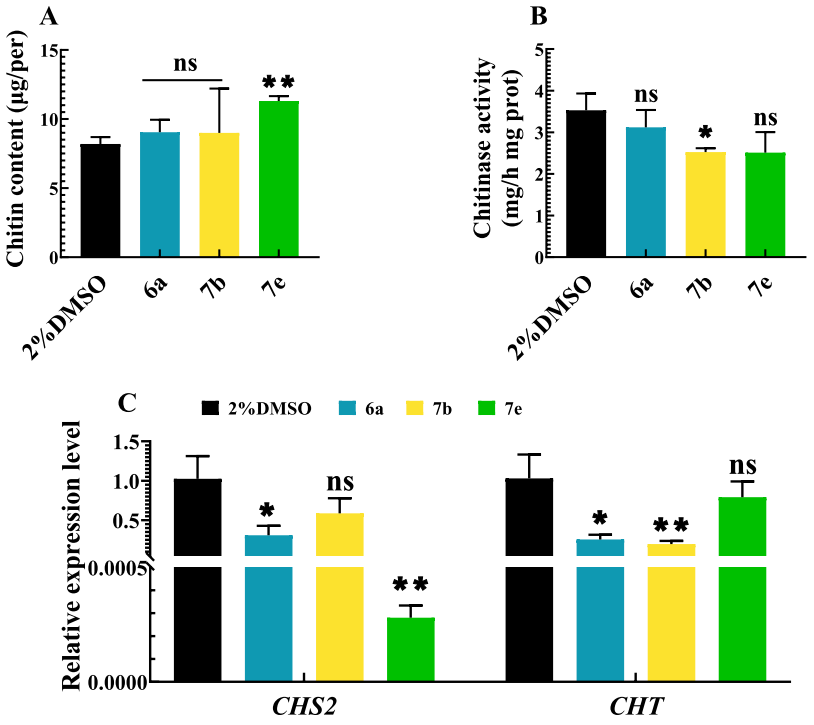


Figure 3. Effects of trehalase (TRE) inhibitors on chitin content, Chitinase activity, and the expression of *CHS2* and *CHT* in *S. frugiperda* after 48 h of treatment. (A) Chitin content and (B) Chitinase activity. (C) Relative expression levels of *CHS2* and *CHT* genes. Values are presented as the means ± SE $*p < 0.05$ and $**p < 0.01$ denote significant differences. ns: not significant (independent samples; *t* test); *CHS2*: chitin synthase 2; *CHT*: Chitinase; 2% DMSO: negative control group; 6a, 7b, and 7e: treatment groups.

The resulting precipitate was resuspended in 200 μL of McIlvaine's buffer. To hydrolyze chitin, 10 μL of Chitinase from *Streptomyces griseus* was added, and the mixture was incubated at 37 °C and 150 rpm in a shaker for 72 h.

Once the hydrolysis reaction was complete, the samples were centrifuged at 12,000 × *g* for 1 min at 25 °C. The supernatant (60 μL) was collected, and an equal volume of sodium borate was added to the supernatant. The mixture was stirred and incubated in a water bath (99.9 °C) for 10 min, followed by cooling to 25 °C. Subsequently, 600 μL of 1 × DMAB was added, and the reaction occurred in a water bath at 37 °C for 20 min. Samples (200 μL) were added to the wells of enzyme-linked plates. The absorbance of the samples was determined to be 585 nm. The absorbance measurements of the standard solution were used to generate a standard curve. Chitin concentration in the sample was calculated based on the standard curve. This procedure was adapted from the determination of chitin content by Yao and Su.[49,50]

2.7. Determination of Chitinase Activities. Larvae were collected in EP tubes after 48 h of treatment, and each experiment included three biological replicates and three technical replicates. Approximately 0.1 g of tissue was homogenized in 1 mL of ice-cold extraction solution and centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant (400 μL) was collected on ice and subjected to chitinase activity testing using a chitinase reagent kit (Suzhou Keming Biotechnology Co., Ltd.). Chitinase activity (mg/h/g fresh weight) was calculated using the formula

$$\begin{aligned}\text{Chitinase activity} &= (\Delta A + 0.2753) \div 6.4108 \times V_{\text{total reaction system}} \\ &\div (V_{\text{sample}} \div V_{\text{total sample}} \times W) \div T \times 10 \\ &= 3.899 \times (\Delta A + 0.2753) \div W\end{aligned}$$

A, absorbance; $\Delta A = A_{\text{measured value}} - A_{\text{control value}}$; *W*, sample weight.

2.8. Observation of the Feeding, Growth, and Development of Treated *S. frugiperda* Larvae. On the first day of the third instar, larvae were selected and individually fed an artificial diet in a feeding box. The food quantity was increased as the larvae aged. Fresh food was provided every 2 days, and larvae excrement was removed to maintain a clean growth environment. The shells were used to determine the larval age and were removed after data were recorded to prevent confusion. The weight and the mortality rate were recorded, and the emergence rate of larvae every 24 h was observed in their phenotype after pupation at the same time. This process continued until the larvae pupated.

Starting from the first day after injecting TRE inhibitors, the larvae were observed daily to record their growth and development. Once pupae were formed, their weights were measured, and the survival, deformity, pupation, and emergence rates of the larvae were recorded. A Canon EOS 50D was used to capture images of abnormal larvae to observe phenotypic changes, and they were categorized into lethal and nonlethal phenotypes based on the lethality of the observed abnormalities.

2.9. Quantification and Statistical Analyses. Statistical analyses were performed using IBM SPSS Statistics 20 software. Data were evaluated for normality and homogeneity of variance (Sharpiro-wilk Test). One-way analysis of variance (ANOVA) or Student's *t* test was used to compare differences between the control and treatment groups. Posthoc tests were conducted using the Tukey. A statistical significance was set at $p < 0.05$. The data were expressed as mean ± standard error. GraphPad Prism software (version 9.0) was used to generate graphs illustrating the analyzed data.

3. RESULTS

3.1. Thiazolidinone Compounds Inhibit Trehalase Activity and Affect Carbohydrate Content in *S. frugiperda* Larvae. Thiazolidinones (6a, 7b, and 7e) with a piperine skeleton (2×10^{-3} mmol/mL) were injected into the third instar *S. frugiperda* larvae and samples were analyzed for the detection of TRE activity after 48 h. The results showed that 6a, 7b, and 7e inhibited TRE1 and TRE2 activity compared with the control group (Figure 2A, B). Among these compounds, 7e exhibited the most potent inhibitory effect.

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

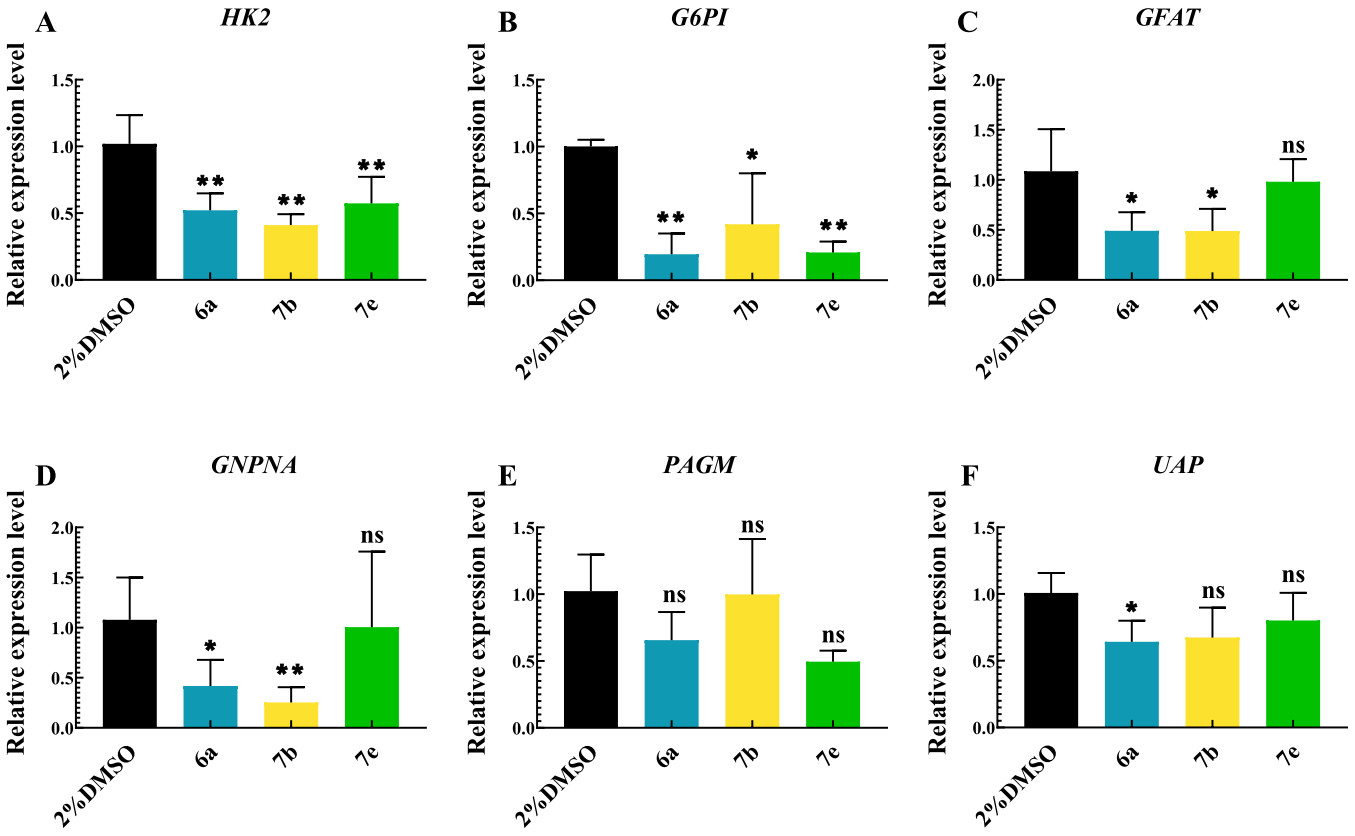


Figure 4. Effects of trehalase (TRE) inhibitors on the expression of genes associated with the chitin synthesis pathway after 48 h of treatment. Relative expression levels of (A) hexokinase 2 (*HK2*), (B) glucose-6-phosphate isomerase (*G6PI*), (C) fructose-6-phosphate transaminase (*GFAT*), (D) glucosamine-phosphate N-acetyltransferase (*GNPNA*), (E) phosphor acetylglucosamine mutase (*PAGM*), and (F) UDP-N-acetylglucosamine pyrophosphorylase (*UAP*). Values are presented as the means ± SE $*p < 0.05$ and $**p < 0.01$ denote significant differences. ns: not significant (independent samples *t* test); 2% DMSO: negative control group; 6a, 7b, and 7e: treatment groups.

Table 4. Developmental Durations of Each Instar in *S. frugiperda* after Injection of Inhibitors[a]

treatment	4th instar	5th instar	6th instar	prepupation	pupation	overall developmental duration
2% DMSO	1.85 ± 0.20 [a]	1.80 ± 0.09 [b]	3.14 ± 0.82 [a]	1.18 ± 0.17 [a]	7.51 ± 0.16 [b]	15.46 ± 0.74 [b]
6a	2.11 ± 0.79 [a]	2.16 ± 0.14 [ab]	3.39 ± 0.36 [a]	1.51 ± 0.44 [a]	8.52 ± 0.42 [a]	18.05 ± 1.07 [a]
7b	1.74 ± 0.19 [a]	2.25 ± 0.22 [ab]	3.71 ± 0.09 [a]	1.30 ± 0.06 [a]	8.49 ± 0.31 [a]	17.33 ± 0.35 [ab]
7e	1.93 ± 0.07 [a]	2.56 ± 0.17 [a]	3.25 ± 0.15 [a]	1.44 ± 0.03 [a]	8.49 ± 0.24 [a]	16.86 ± 0.53 [ab]

[a]Durations are presented in days. Superscript letters denote significant differences ($p < 0.05$) from the control group. The analysis included comparisons among the four different treatments within the same *S. frugiperda* larvae. Values are presented as means ± SE (ANOVA followed by Tukey's post hoc test). 2% DMSO: negative control group; 6a, 7b, and 7e: treatment groups.

These results demonstrated that these three types of TRE inhibitors can be used in follow-up experiments. Further testing of the changes in carbohydrate content revealed that 6a had no effect, whereas 7b and 7e significantly increased the glucose and glycogen contents (Figure 2C–E). The expression levels of the *TRE1* and *TRE2* genes in *S. frugiperda* larvae were significantly decreased after injection with 6a, 7b, and 7e, compared to the control group (Figure 2F).

3.2. Thiazolidinone Compounds Affect Chitinase Activity and Chitin Content in *S. frugiperda* Larvae. The chitin content in the 7e group was significantly higher than that in the control group, whereas there was no significant difference between the control and the 6a and 7b groups (Figure 3A). Compound 7b significantly reduced the Chitinase activity of *S. frugiperda* larvae; however, compounds 6a and 7e had no effect on Chitinase activity (Figure 3B). The expression levels of *CHS2* were significantly down-regulated in the 6a and 7e groups. Moreover, the expression levels of *CHT* were significantly decreased in the 6a and 7b groups (Figure 3C).

3.3. Thiazolidinone Compounds Decrease the Expression of Chitin Metabolism-Related Genes in *S. frugiperda*. We investigated changes in the expression of the key genes involved in chitin synthesis. The results showed that the expression of *HK2, G6PI, GFAT, GNPNA*, and *UAP* was significantly decreased after injection with 6a, similar to that in the group treated with 7b, except for *UAP* (Figure 4). In contrast, inhibitor 7e significantly decreased the expression of only *HK2* and *G6PI* genes (Figure 4A and 4B).

3.4. Thiazolidinone Compounds Affect the Development *S. frugiperda* Larvae. The developmental time of the fourth and sixth instar larvae and prepupation of in *S. frugiperda* showed no significant differences among the groups. However, compared to the control group, the development time of fifth instar larvae was significantly increased in the 7e group, and the development time of pupae was prolonged by

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

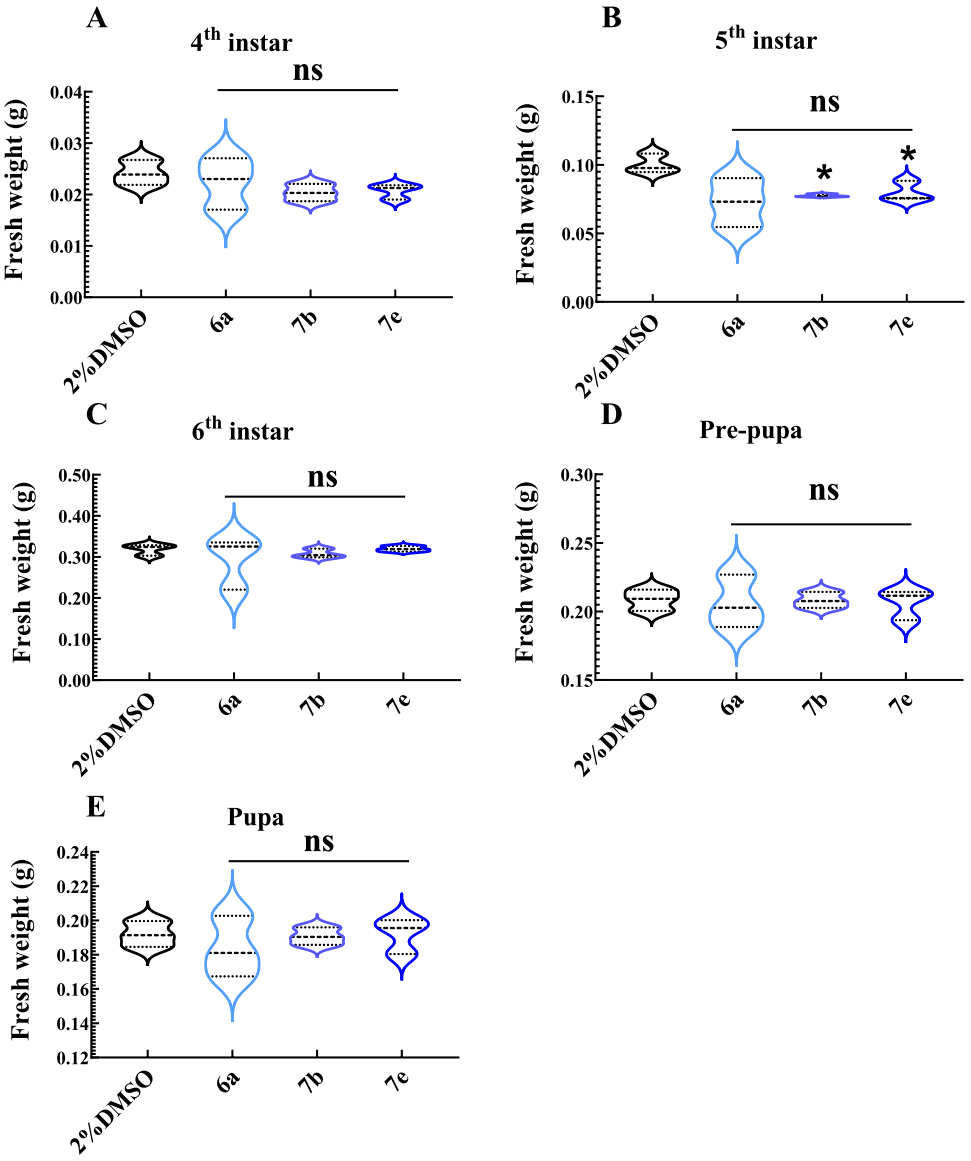


Figure 5. Effect of trehalase (TRE) inhibitors on the weight of each instar in *S. frugiperda*. (A) Fourth, (B) fifth, and (C) sixth instar. (D) Prepupa and (E) pupa stages. Values are presented as the means ± SE *$p < 0.05$ denotes significant differences. ns: not significant (independent samples; *t* test); 2% DMSO: negative control group; 6a, 7b, and 7e: treatment groups.

all three inhibitors. Overall, 6a, 7b, and 7e extended the development time of larvae, with 6a having the most significant effect compared with the control group (Table 4).

Weight changes in *S. frugiperda* at each developmental stage, from the fourth instar to the pupal stage, were assessed. The results showed that there were no significant differences in weight between the fourth and sixth instar larvae (Figure 5A, C). The weight of the 6a group was significantly lower than that of the control group, but there were no differences in the 7b and 7e groups among the fourth instar larvae (Figure 5B). No significant differences were observed in the prepupation and pupation weights between the groups (Figure 5D,E).

3.5. Thiazolidinone Compounds Decrease the Survival Rate of *S. frugiperda* Larvae. In the control group, the survival rate decreased from the third instar to the fifth instar, reaching 65.17% in the first instar (Figure 6A). This decline in survival rate gradually continued from the fifth instar to adulthood, with 41.57% of the initially treated (on the first day of the third instar) larvae successfully emerging as adult moths. The death of larvae in the 7b and 7e groups mainly occurred between the fourth and fifth instars, resulting in an emergence rate of less than half compared to that at the initial treatment (Figure 6A). The survival rates of emergence were 48.86 and 40.44% in groups 7b and 7e, respectively, which were lower compared with those of the control group (Figure 6A). Notably, larvae in the 6a group experienced high mortality rates between the third and fifth instars, resulting in a survival rate of only 28.89% by the fifth instar. Moreover, larvae in the 6a group that successfully emerged into adult moths accounted for only 20.00% of the initially treated larvae, which was significantly lower compared to the 7b, 7a, and control groups (Figure 6A). Although larval mortality was higher in the treated groups, particularly in the 6a group, during the larval stage, there were no significant differences in the pupation and emergence rates among the groups (Figure 6B and 6C).

3.6. Thiazolidinone Compounds Cause Abnormal Phenotypes and Increase Deformity Rates in *S.*

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

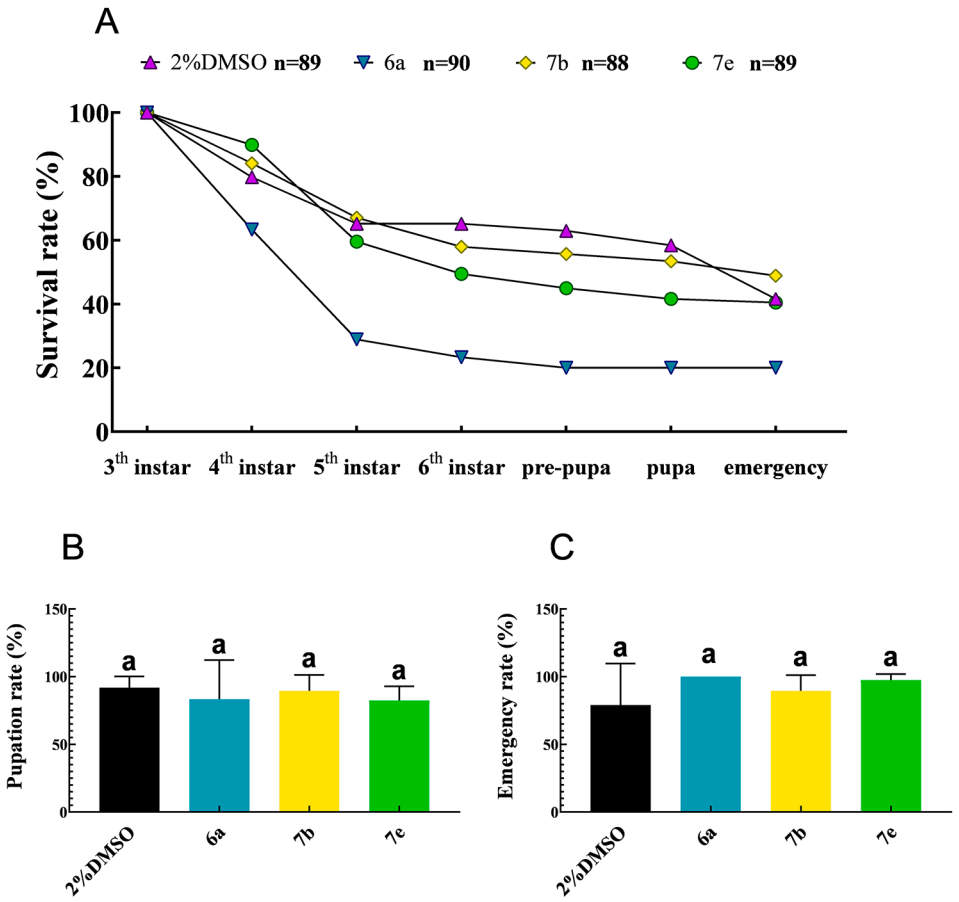


Figure 6. Effect of trehalase (TRE) inhibitors on the survival, pupation, and emergence rates of each instar in *S. frugiperda*. (A) Survival, (B) pupation, and (C) emergence rates. Values are presented as the means ± SE. Pupation rate = (pupal number/sixth instar larvae number) × 100%; emergence rate = (number of adults/pupal number) × 100%. Values are presented as the means ± SE ns: not significant (independent samples; *t* test); 2% DMSO: negative control group; 6a, 7b, and 7e: treatment groups.

***frugiperda* Larvae.** As shown in Figure 7, abnormal phenotypes of larvae were observed in groups 6a, 7b, and 7e during development, and the deformity rates were significantly higher than those in the control group (Table 5). Larval mortality due to molting failure in groups 6a, 7b, and 7e, compared to normally developing larvae, was mainly observed in the third, fourth, and fifth instars, resulting in what was termed an abnormal lethal phenotype (Figure 7A). Additionally, larvae in the 6a, 7b, and 7e groups exhibited a certain abdominal segment contraction phenotype, resembling the loss of the outer epidermis. This phenotype appeared as early as the fourth instar and was categorized as an abnormal nonlethal phenotype (Figure 7B). Notably, this phenotype persisted into

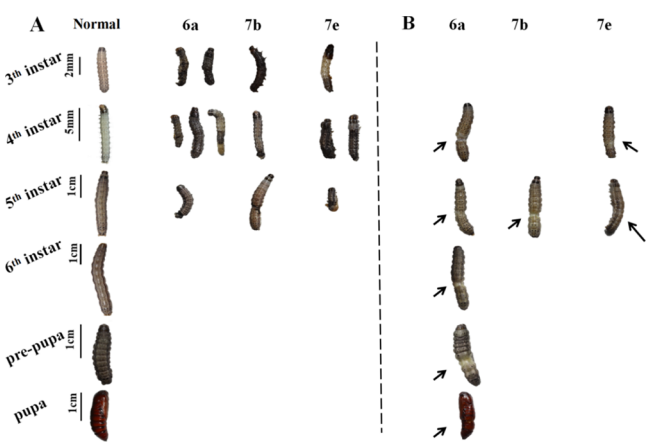


Figure 7. Effect of trehalase (TRE) inhibitors on the phenotype of *S. frugiperda* larvae. (A) Abnormal death phenotype, and (B) abnormal nondeath phenotype. The same larvae were tracked to observe abnormal nondeath phenotypes across different treatments. The number of deaths during development was counted and all abnormal death phenotypes and abnormal nondeath phenotype were recorded. Values are presented as the means ± SE (independent samples; *t* test). 6a, 7b, and 7e: treatment groups.

Table 5. Abnormality Rates in Each Instar of *S. frugiperda* after Injection of Three Trehalase Inhibitors[a]

treatment	number of larvae (*n*)	abnormality rate (%)	*p*-values
2% DMSO	88	1.14	
6a	90	14.44	$p = 0.001^b$
7b	89	12.36	$p = 0.003^b$
7e	90	12.22	$p = 0.003^b$

[a]Comparisons were made between the four different treatments using the same *S. frugiperda* larvae. Superscript denote significant differences ($p < 0.05$) from the control group (chi-square test). Values are presented by mean ± SE (ANOVA followed by Tukey's post hoc test). 2% DMSO: negative control group; 6a, 7b, and 7e: treatment groups.

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

the subsequent instar during development in the 6a group. Larvae with this phenotype were capable of molting normally and completing their development. The larvae in groups 7b and 7e also exhibited this phenotype, although it was not observed until the sixth instar.

4. DISCUSSION

Trehalases are unique and highly conserved enzymes found in bacteria, fungi, plants, and certain invertebrates.[51] They have been recognized as a novel target for pest control because they are the sole enzymes responsible for trehalose hydrolysis, a crucial step in chitin biosynthesis.[52] Several TRE inhibitors have been extensively studied as potential insecticides.[32,41,53] However, some TRE inhibitors exhibit different affinities for TREs from different species.[54,55] For example, 5-thiotrehalose is structurally more similar to TRE than validamycin A, which effectively inhibits trehalose utilization in *Clostridium difficile*. However, it lacks activity against *Aedes aegypti*.[55,56] Halogenin acts as a competitive inhibition of TREs, and the dissociation of the enzyme−inhibitor complex is notably slow.[57] Furthermore, numerous studies have confirmed that validamycin and validamycin A, exhibit similar inhibitory effects on Lepidoptera, Diptera, Hemiptera, Coleoptera, and other insects.[51,56,58−61]

In this study, we injected larvae with compounds 6a, 7b, and 7e and found that all three inhibitors have a strong affinity for TRE in *S. frugiperda*, enabling them to inhibit TRE activity in larvae after 48 h of treatment (Figure 2A,B). Furthermore, all three inhibitors significantly downregulated the expression of *TRE1* and *TRE2* genes (Figure 2F), demonstrating that they consistently inhibited TRE at the protein and molecular levels (Figure 2). These results underscore the potential of piperine derivatives and thiazolidone as TRE inhibitors.[39−41]

Previous studies have shown that the inhibition of TRE activity hinders the decomposition of trehalose in insects, resulting in increased trehalose content and decreased glucose content, ultimately disrupting normal glucose metabolism.[51,60] Glycogen is primarily synthesized from UDP glucose through glycogen synthase (GS),[62] which mainly originates from dietary carbohydrates and glucose.[63] Inhibitors 7b and 7e increased glucose and glycogen concentrations after suppressing TRE activity (Figure 2C−E). In contrast, the glycogen, trehalose, and glucose contents remained unchanged after injection with ZK-PI-5 and ZK-PI-9,[40] suggesting that 7b and 7e may employ alternative mechanisms to promote glucose and glycogen synthesis.

Chitin is predominantly found in invertebrates, insects, marine diatoms, algae, fungi, and yeast. In insects, it serves as the main component of the cuticle, offering protection against dehydration and mechanical damage, and acts as a physical barrier against predation.[64,65] During insect development and metamorphosis, eight enzymes are involved in the chitin biosynthesis pathway, with fluctuations in chitin content linked to chitin synthases and Chitinase.[27] Silencing the chitin synthase gene in *Anopheles gambiae* reduces the transcription level of chitin synthase 1 by 62.8% and the chitin content by 33.8%.[66,67] Insect growth regulators can disrupt insect molting and the stratum corneum, hindering the development of beneficial arthropods.[12,69] During chitin degradation, molting fluid, rich in Chitinase, accumulates between the old and new stratum corneums to assist in larval and pupal molting. The three TRE inhibitors, 6a, 7b, and 7e, downregulated the expression of *HK2* and *G6PI* in the chitin synthesis pathway (Figure 4), whereas 6a and 7b significantly reduced the expression of *CHT*, *GFAT*, and *GNPNA*. Treating larvae with 1 μg/μL Validamycin inhibits TRE activity, stimulating the upregulation of *SfGFAT*, *SfCHT*, and *SfGNA* to maintain chitin content. These findings confirm the important roles of *GFAT* and *GNA* in the feedback regulatory mechanism of chitin biosynthesis.[32] The three TRE inhibitors, 6a, 7b, and 7e, may severely disrupt the balance of chitin metabolism in insects, with 7b inhibiting Chitinase activity and 7e increasing the chitin content (Figure 3).

Furthermore, the growth, development, and reproduction of invasive insects are influenced by various biological and abiotic factors.[16,68] The survival rate of emerging *S. frugiperda* larvae was less than 50% (Figure 6A) following treatment with larvae 6a, 7b, and 7e, primarily owing to abnormal chitin metabolism. Previous studies have shown that *GFAT* and *GNAPNA* play regulatory roles in chitin biosynthesis.[42,69,70] For instance, when *Litopenaeus vannamei* is exposed to alkaline pH conditions and cadmium stress, *LvGFAT* is strongly expressed in the liver, indicating its importance in environmental stress resistance.[71] Inhibition of *GFAT* expression can significantly increase the deformity and mortality rates in *Nilaparvata lugens*.[72] Bolognesi et al.[73] reported that *SfCHSB* and *SfCHI* are highly expressed in the midgut epithelium. When *SfCHII* is expressed, there is little or no chitin in the midgut, indicating that *SfCHI* primarily degrades chitin in the peritrophic membrane (PM). Silencing of *UAP* can lead to the death of over half of *Bactrocera Dorsalis* and larvae because of abnormal phenotypes.[74] Injecting dsApis*CHS* into *Acyrthosiphon pisum* increases the malformation rate of early nymphs to 44%, and knocking out the *TRE* gene leads to high mortality rates in fruit flies and *Tribolium castaneum*.[75,76]

The TRE inhibitors injected (1 μg/L) in this study significantly increased the malformation rate of the larvae. The results showed that 6a had the most significant impact on the development of *S. frugiperda* larvae, significantly reducing the weights of the fourth, fifth, and sixth instar larvae (Figure 5). Mortality resulting from molting deformities was mainly concentrated in the third, fourth, and fifth instars, leading to a sharp decrease in the survival rates of the third to fourth and fourth to fifth instar larvae in the 6a group (Figure 6A). Zhong et al.[40] found that injection of the TRE inhibitors ZK-PI-5 and ZK-PI-9 had no significant effect on the growth and development of *S. frugiperda* from the fourth instar to the pupal stage. However, the proportion of abnormal phenotypes characterized by failed emergence increased, potentially owing to the contraction of the abdominal segment in the larvae caused by the loss of the outer epidermis. *HK* is a key regulatory factor in insect development, and its expression and activity levels of *HK* in diapause pupae are lower than those in nondiapause pupae. Downregulation of *HK* activity reduces cell viability and delays pupal development by decreasing metabolic activity and increasing the activity of reactive oxygen species (ROS).[77] All three TRE inhibitors significantly reduced *HK2* expression (Figure 4A), which may be the main reason for the prolonged pupal stages. Cnsoli et al.[78] found that the pupa of *Trichogramma previosum* is more sensitive to pesticides than eggs, larvae, or prepupae. Some chemical pesticides can prolong the pupal stage of parasitic wasps, possibly due to physiological homeostatic overcompensation mechanisms produced by the body.[79]

S. frugiperda exhibits strong adaptability to host plants during outbreaks.[80,81] All three TRE inhibitors 6a, 7b, and 7e,

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

were avirulent in late larvae (sixth instar-prepupa, prepupa-pupa) and had no effect on pupation or emergence rates (Figure 6), indicating that late larvae adapted to the TRE inhibitors, and prolonged pupation reflects their adaptability. Notably, in addition to the abnormal lethal phenotype, *S. frugiperda* larvae treated with the TRE inhibitors also exhibited nonlethal abnormal phenotypes. Investigating the specific mechanisms behind these phenomena will require time and a combination of technologies, such as RNAi.[68,82]

In conclusion, trehalose metabolism and chitin biosynthesis pathways are important physiological processes in insects, profoundly influencing survival and normal development when inhibited. Our findings demonstrate that the thiazolidinone compounds 6a, 7b, and 7e, play significant roles in the teratogenicity and mortality of larvae. All three inhibitors have the potential to delay the growth and development of *S. frugiperda* larvae, with 6a exhibiting the most pronounced lethal effect. Therefore, these compounds hold significant promise as environmentally friendly insecticides with potential applications in pest control strategies.

AUTHOR INFORMATION

Corresponding Authors

Hongxia Duan − *Innovation Center of Pesticide Research, Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100193, China;* orcid.org/0000-0003-2516-854X; Email: hxduan@cau.edu.cn

⊥**Yan Wu** − *Key Laboratory of Surveillance and Management of Invasive Alien Species, Guizhou Education Department, Department of Biology and Engineering of Environment, Guiyang University, Guiyang 550005, China;* Email: 15150535703@163.com

Authors

Bin Tang − *College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China*

Shangrong Hu − *College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China*

Yujia Luo − *College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China*

Dongmei Shi − *Innovation Center of Pesticide Research, Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100193, China*

Xiangyu Liu − *College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China; Key Laboratory of Surveillance and Management of Invasive Alien Species, Guizhou Education Department, Department of Biology and Engineering of Environment, Guiyang University, Guiyang 550005, China*

Fan Zhong − *College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China*

Xinyi Jiang − *College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China*

Gao Hu − *College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China*

Can Li − *Key Laboratory of Surveillance and Management of Invasive Alien Species, Guizhou Education Department, Department of Biology and Engineering of Environment, Guiyang University, Guiyang 550005, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.3c08898

Author Contributions

B.T.: Conceptualization, Supervision, Writing—original draft, Writing—review and editing. S.H.: Investigation, Writing—original draft, Writing—review and editing. Y.L.: Methodology, Investigation, Visualization, Writing—original draft. D.S.: Visualization, Writing—review and editing. X.L.: Methodology, Writing—review and editing. F.Z.: Methodology, Investigation. X.J.: Methodology, Visualization. G.H.: Supervision. C.L.: Supervision. H.D.: Conceptualization, Supervision. Y.W.: Conceptualization, Supervision.

Notes

The authors declare no competing financial interest.

⊥Lead contact.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program of China (Grant No. 2022YFD1700200), the Sixth Batch of Guizhou Province High-level Innovative Talent Training Program [2022], the Guiyang Science and Technology Personnel Training Project [2022]43-16, the Program for Natural Science Research in Guizhou Education Department QJJ[2023]024, Guizhou Provincial Science and Technology Project for Academic New Seedling Cultivation and Free Exploration Innovation and Guiyang Training Project for "Thousand Level" Talents in Guizhou Province.

REFERENCES

(1) Sparks, A. N. A Review of the biology of the fall armyworm. *FLA. ENTOMOL.* **1979**, *62*, 82.

(2) Hou, Y. Y.; Ma, Y.; Xu, W.; et al. *Spodoptera frugiperda* egg mass scale thickness modulates *Trichogramma* parasitoid performance. *Entomol. Gen.* **2022**, *42*, 589−596.

(3) Kenis, M.; Benelli, G.; Biondi, A.; et al. Invasiveness, biology, ecology, and management of the fall armyworm, *Spodoptera frugiperda*. *Entomol. Gen.* **2023**, *43*, 187−241.

(4) Pashley, D. P.; Johnson, S. J.; Sparks, A. N. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Amer.* **1985**, *78*, 756−762.

(5) Pashley, D. P. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? *Ann. Entomol. Soc. Amer.* **1986**, *79*, 898−904.

(6) Pashley, D. P.; Martin, J. A. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Amer.* **1987**, *80*, 731−733.

(7) Goergen, G.; Kumar, P. L.; Sankung, S. B.; et al. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in west and central Africa. *PLoS One.* **2016**, *11*, No. e0165632.

(8) Zhou, Y.; Wu, Q. L.; Zhang, H. W.; et al. Spread of invasive migratory pest Spodoptera frugiperda and management practices throughout China. *J INTEGR AGR.* **2021**, *20*, 637−645.

(9) Wu, D. H.; Li, Y. R.; Wang, S. Q.; et al. Effects of *Zea mays* and three weeds around *Z. mays* fields on the growth, development and oviposition of *Spodoptera frugiperda*. *Plant Prot.* **2021**, *47*, 116−121.

(10) Yu, S. J. Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). *PESTIC BIOCHEM PHYS.* **1991**, *39*, 84−91.

(11) Li, R.; Zhu, B.; Liang, P.; et al. Identification of carboxylesterase genes contributing to multi-insecticide resistance in *Plutella xylostella* (L.). *Entomol. Gen.* **2022**, *6*, 967−976.

(12) Desneux, N.; Decourtye, A.; Delpuech, J. M. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* **2007**, *52*, 81−106.

(13) Zhu, Y. C.; Blanco, C. A.; Portilla, M.; et al. Evidence of multiple/cross resistance to Bt and organophosphate insecticides in

Puerto Rico population of the fall armyworm, *Spodoptera frugiperda*. *Pestic. Biochem. Physiol.* **2015**, *122*, 15−21.
(14) Wang, P.; Granados, R. R. Molecular structure of the peritrophic membrane, (PM): identification of potential PM target sites for insect control. *Arch. Insect Biochem. Physiol.* **2021**, *47*, 110−118.
(15) Muraro, D. S.; de Neto, D. O. A.; Kanno, R. H.; et al. Inheritance patterns, cross-resistance and synergism in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistant to emamectin benzoate. *Pest Manag. Sci.* **2021**, *77*, 5049−5057.
(16) Liu, B.; Lu, Y. Y.; Wan, F. H.; et al. Biological invasion of insects: the roles of microbes. *Entomol. Gen.* **2022**, *42*, 851−861.
(17) Wang, X. J.; Tian, L. X.; Ricupero, M.; et al. Hormesis effects of chlorantraniliprole on a key egg parasitoid used for management of rice lepidopterans. *Entomol. Gen.* **2022**, *42*, 941−948.
(18) Elbein, A. D.; Pan, Y. T.; Pastuszak, I.; et al. New insights on trehalose: a multifunctional molecule. *GLYCOBIOLOGY.* **2003**, *13*, 17R−27R.
(19) Walmagh, M.; Zhao, R.; Desmet, T. Trehalose analogues: latest insights in properties and biocatalytic production. *Int. J. Mol. Sci.* **2015**, *16*, 13729−13745.
(20) Oide, S.; Inui, M. Trehalose acts as a uridine 5′-diphosphoglucose-competitive inhibitor of trehalose 6-phosphate synthase in *Corynebacterium glutamicum*. *FEBS. J.* **2017**, *284*, 4298−4313.
(21) Takiguchi, M.; Niimi, T.; Su, Z. H.; et al. Trehalase from male accessory gland of an insect, *Tenebrio molitor*. cDNA sequencing and developmental profile of the gene expression. *Biochem. J.* **1992**, *288*, 19−22.
(22) Su, Z. H.; Ikeda, M.; Sato, Y.; et al. Molecular characterization of ovary trehalase of the silkworm, *Bombyx mori* and its transcriptional activation by diapause hormone. *Biochim. Biophys. Acta* **1994**, *1218*, 366−374.
(23) Mitsumasu, K.; Azuma, M.; Niimi, T.; et al. Membrane-penetrating trehalase from silkworm *Bombyx mori*. molecular cloning and localization in larval midgut. *Insect Mol. Biol.* **2005**, *14*, 501−508.
(24) Lee, J. H.; Saito, S.; Mori, H.; et al. Molecular cloning of cDNA for trehalase from the European honeybee, *Apis mellifera* L., and its heterologous expression in *Pichia pastoris*. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 2256−2265.
(25) Moussian, B. Chitin: structure, chemistry and biology. *Adv. Exp. Med. Biol.* **2019**, *1142*, 5−18.
(26) Merzendorfer, H.; Zimoch, L. Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. *J. Exp. Biol.* **2003**, *206*, 4393−4412.
(27) Zhu, K. Y.; Merzendorfer, H.; Zhang, W. Q.; et al. Biosynthesis, turnover, and functions of chitin in insects. *Annu. Rev. Entomol.* **2016**, *61*, 177−196.
(28) Sode, K.; Akaike, E.; Sugiura, H.; et al. Enzymatic synthesis of a novel trehalose derivative, 3,3′-diketotrehalose, and its potential application as the trehalase enzyme inhibitor. *FEBS. Lett.* **2001**, *489* (1), 42−45.
(29) Liebl, M.; Nelius, V.; Kamp, G.; et al. Fate and effects of the trehalase inhibitor trehazolin in the migratory locust (*Locusta migratoria*). *J. Insect Physiol.* **2010**, *56*, 567−574.
(30) Wegener, G.; Macho, C.; Schlöder, P.; et al. Long-term effects of the trehalase inhibitor trehazolin on trehalase activity in locust flight muscle. *J. Exp. Biol.* **2010**, *213*, 3852−3857.
(31) Iwasa, T.; Higashide, E.; Yamamoto, H.; et al. Studies on validamycins, new antibiotics. II. Production and biological properties of validamycins A and B. *J. Antibiot.* **1971**, *24*, 107−113.
(32) Luo, Y. J.; Chen, Y.; Wang, X. J.; et al. Validamycin affects the development and chitin metabolism in *Spodoptera frugiperda* by inhibiting trehalase activity. *Entomol. Gen.* **2022**, *42*, 931−939.
(33) Kameda, Y.; Asano, N.; Yamaguchi, T.; et al. Validoxylamines as trehalase inhibitors. *J. Antibiot.* **1987**, *40*, 563−565.
(34) Kyosseva, S. V.; Kyossev, Z. N.; Elbein, A. D. Inhibitors of pig kidney trehalase. *Arch. Biochem. Biophys.* **1995**, *316*, 821−826.
(35) Ando, O.; Nakajima, M.; Hamano, K.; et al. Isolation on trehalamine, the aglycon of trehazolin, from microbial broths and characterization of trehazolin related compounds. *J. Antibiot.* **1993**, *46*, 1116−1125.
(36) El Nemr, A.; El Ashry, E. S. H. Potential trehalase inhibitors: syntheses of trehazolin and its analogues. *Adv. Carbohydr. Chem. Biochem.* **2011**, *65*, 45−114.
(37) Vértesy, L.; Fehlhaber, H. W.; Schulz, A. The trehalase inhibitor salbostatin, a novel metabolite from *Streptomyces albus*, ATCC21838. *Angew. Chem., Int. Ed.* **1994**, *33*, 1844−1846.
(38) Han, Q.; Wu, N.; Li, H. L.; et al. A piperine-based scaffold as a novel starting point to develop inhibitors against the potent molecular target *Of* ChtI. *J. Agric. Food Chem.* **2021**, *69* (27), 7534−7544.
(39) Wang, S. T.; Chen, Y.; Luo, Y. J.; et al. Effect of three novel compounds on trehalose and chitin metabolism and development of *Spodoptera frugiperda*. *Sci. Agric. Sin.* **2022**, *55*, 1568−1578.
(40) Zhong, F.; Yu, L. H.; Jiang, X. Y.; et al. Potential inhibitory effects of compounds ZK-PI-5 and ZK-PI-9 on trehalose and chitin metabolism in *Spodoptera frugiperda* (J. E. Smith). *Front. Physiol.* **2023**, *14*, No. 1178996.
(41) Jiang, X. Y.; Zhong, F.; Chen, Y.; et al. Novel compounds ZK-PI-5 and ZK-PI-9 regulate the reproduction of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), with insecticide potential. *J. Econ. Entomol.* **2023**, *116*, 1850.
(42) Zhang, J. Y.; Han, Q.; Jiang, Z. Y.; et al. Chitinase inhibitors and synthesis and agricultural bioactivity of thiazolidinones: a review. *Chin. J. Pestic. Sci.* **2021**, *23*, 421−437.
(43) Han, Q.; Wu, N.; Liu, Y. Y.; et al. Piperonyl-tethered rhodanine derivatives potently inhibit chitinolytic enzymes of *Ostrinia furnacalis*. *J. Agric. Food Chem.* **2022**, *70* (24), 7387−7399.
(44) Duan, H. X.; Han, Q.; Yang, Q. Compound with piperine skeleton structure, preparation and application thereof. CN111269220A[P]. 2020-06−12.
(45) Jiang, Z.; Shi, D.; Li, H.; et al. Rational design and identification of novel piperine derivatives as multichitinase inhibitors. *J. Agric. Food Chem.* **2022**, *70* (33), 10326−10336.
(46) Pinto, J.; Torres, A. F.; Truzi, C. C.; et al. Artificial corn-based diet for rearing *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J INSECT SCI.* **2019**, *19* (4), 2.
(47) Gurusamy, D.; Mogilicherla, K.; Shukla, J. N.; et al. Lipids help double-stranded RNA in endosomal escape and improve RNA interference in the fall armyworm, *Spodoptera frugiperda*. *Arch. Insect Biochem. Physiol.* **2020**, *104*, No. e21678.
(48) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* **2001**, *25*, 402−408.
(49) Yao, Q.; Zhang, D.; Tang, B.; et al. Identification of 20-hydroxyecdysone late-response genes in the chitin biosynthesis pathway. *PloS one.* **2010**, *5*, No. e14058.
(50) Su, C. C. *Analysis of Chitinase gene family and chitin concentration in striped rice borer, Chilo suppressalis[MS]*; Nanjing Agricultural University, 2017.
(51) Tang, B.; Yang, M. M.; Shen, Q. D.; et al. Suppressing the activity of trehalase with validamycin disrupts the trehalose and chitin biosynthesis pathways in the rice brown planthopper, *Nilaparvata lugens*. *Pestic. Biochem. Physiol.* **2017**, *137*, 81−90.
(52) Tang, B.; Zhang, L.; Xiong, X. P.; et al. Advances in trehalose metabolism and its regulation of insect chitin synthesis. *Sci. Agri. Sin.* **2018**, *51*, 697−707.
(53) Asano, N. Glycosidase inhibitors: update and perspectives on practical use. *GLYCOBIOLOGY.* **2003**, *13*, 93R−104R.
(54) Shukla, E.; Thorat, L. J.; Nath, B. B.; et al. Insect trehalase: physiological significance and potential applications. *GLYCOBIOLOGY.* **2015**, *25*, 357−367.
(55) Danielson, N. D.; Collins, J.; Stothard, A. I.; et al. Degradation-resistant trehalose analogues block utilization of trehalose by hypervirulent *Clostridioides difficile*. *Chem. Commun (Camb).* **2019**, *55*, 5009−5012.

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433

(56) Marten, A. D.; Stothard, A. I.; Kalera, K.; et al. Validamycin A delays development and prevents flight in *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* **2020**, *57*, 1096−1103.
(57) Ando, O.; Nakajima, M.; Kifune, M.; et al. Trehazolin, a slow tight-binding inhibitor of silkworm trehalase. *Biochim. Biophys. Acta* **1995**, *1244*, 295−302.
(58) Tatun, N.; Wangsantitham, O.; Tungjitwitayakul, J.; et al. Trehalase activity in fungus-growing termite, *Odontotermes feae* (Isoptera: Termitideae) and inhibitory effect of validamycin. *J. Econ. Entomol.* **2014**, *107*, 1224−1232.
(59) Tatun, N.; Tungjitwitayakul, J.; Sakurai, S. Developmental and lethal effects of trehalase inhibitor (validamycin) on the *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Ann. Entomol. Soc. Amer.* **2016**, *109*, 224−231.
(60) Yu, H. Z.; Huang, Y. L.; Lu, Z. J.; et al. Inhibition of trehalase affects the trehalose and chitin metabolism pathways in *Diaphorina citri* (Hemiptera: Psyllidae). *Insect Sci.* **2021**, *28*, 718−734.
(61) Li, Y.; Xu, Y. H.; Wu, S. J.; et al. Validamycin inhibits the synthesis and metabolism of trehalose and chitin in the oriental fruit fly, *Bactrocera dorsalis* (Hendel). *INSECTS.* **2023**, *14*, 671.
(62) Zhang, L.; Wang, H. J.; Chen, J. Y.; et al. Glycogen phosphorylase and glycogen synthase: gene cloning and expression analysis reveal their role in trehalose metabolism in the brown planthopper, *Nilaparvata lugens Stål* (Hemiptera: Delphacidae). *J. Insect Sci.* **2017**, *17*, 42.
(63) Thompson, S. N. Trehalose - the insect 'blood' sugar. *Adv. Insect Physiol.* **2008**, *31*, 205−228.
(64) Synowiecki, J.; Al-Khateeb, N. A. Production, properties, and some new applications of chitin and its derivatives. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 145−171.
(65) Muthukrishnan, S.; Mun, S.; Noh, M. Y.; et al. Insect cuticular chitin contributes to form and function. *Curr. Pharm. Des.* **2020**, *26*, 3530−3545.
(66) Zhang, X.; Zhang, J.; Zhu, K. Y. Chitosan/double-stranded RNA nanoparticle-mediated RNA interference to silence chitin synthase genes through larval feeding in the African malaria mosquito (*Anopheles gambiae*). *Insect Mol. Biol.* **2010**, *19*, 683−693.
(67) Dhadialla, T. S.; Carlson, G. R.; Le, D. P. New insecticides with ecdysteroidal and juvenile hormone activity. *Annu. Rev. Entomol.* **1998**, *43*, 545−569.
(68) Zhu, K. Y.; Palli, S. R. Mechanisms, applications, and challenges of insect RNA interference. *Annu. Rev. Entomol.* **2020**, *65*, 293−311.
(69) Liu, X. J.; Zhang, J. Z.; Zhu, K. Y. Chitin in Arthropods: Biosynthesis, Modification, and Metabolism. *Adv. Exp. Med. Biol.* **2019**, *1142*, 169−207.
(70) Kato, N.; Dasgupta, R.; Smartt, C. T. Glucosamine: fructose-6-phosphate aminotransferase: gene characterization, chitin biosynthesis and peritrophic matrix formation in *Aedes aegypti*. *Insect Mol. Biol.* **2002**, *11*, 207−216.
(71) Liu, Y.; Cai, D. X.; Wang, L.; et al. Glucosamine: fructose-6-phosphate amidotransferase in the white shrimp *Litopenaeus vannamei*: characterization and regulation under alkaline and cadmium stress. *ECOTOXICOLOGY.* **2015**, *24*, 1754−1764.
(72) Xu, C. D.; Liu, Y. K.; Qiu, L. Y.; et al. GFAT and PFK genes show contrasting regulation of chitin metabolism in *Nilaparvata lugens*. *Sci Rep.* **2021**, *11*, 5246.
(73) Bolognesi, R.; Ribeiro, A. F.; Terra, W. R.; et al. The peritrophic membrane of *Spodoptera frugiperda*: secretion of peritrophins and role in immobilization and recycling digestive enzymes. *Arch. Insect Biochem. Physiol.* **2001**, *47*, 62−75.
(74) Yang, W. J.; Wu, Y. B.; Chen, L.; et al. Two chitin biosynthesis pathway genes in *Bactrocera dorsalis* (Diptera: Tephritidae): molecular characteristics, expression patterns, and roles in larval-pupal transition. *J. Econ. Entomol.* **2015**, *108*, 2433−2442.
(75) Tang, B.; Wei, P.; Zhao, L. N.; et al. Knockdown of five trehalase genes using RNA interference regulates the gene expression of the chitin biosynthesis pathway in *Tribolium castaneum*. *BMC Biotechnology.* **2016**, *16*, 67.
(76) Ye, C.; Jiang, Y. D.; An, X.; et al. Effects of RNAi-based silencing of chitin synthase gene on moulting and fecundity in pea aphids, (*Acyrthosiphon pisum*). *Sci. Rep.* **2019**, *9*, 3694.
(77) Lin, Y.; Meng, Y.; Wang, Y. X.; et al. Vitellogenin receptor mutation leads to the oogenesis mutant phenotype ″scanty vitellin″ of the silkworm, *Bombyx mori*. *J. Biol. Chem.* **2013**, *288*, 13345−13355.
(78) Consoli, F. L.; Parra, J. R. P.; Hassan, S. A. Side effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), anatural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *J. Appl. Entomol.* **1998**, *122*, 43−47.
(79) Calabrese, E. J. Hormesis: Why it is important to toxicology and toxicologists. *Environ. Toxicol. Chem.* **2009**, *27*, 1451−1474.
(80) Zhao, S. Y.; Luo, Q. M.; Sun, X. X.; et al. Comparison of morphological and biological characteristics between *Spodoptera frugiperda* and *Spodoptera litura*. *China Plant Prot.* **2019**, *39*, 26−35.
(81) Wang, P.; He, P. C.; Hu, L.; et al. Host selection and adaptation of the invasive pest *Spodoptera frugiperda* to indica and japonica rice cultivars. *Entomol. Gen.* **2022**, *42*, 403−411.
(82) Yan, S.; Yin, M. Z.; Shen, J. Nanoparticle-based non-transformative RNA insecticides for sustainable pest control: mechanisms, current status and challenges. *Entomol. Gen.* **2023**, *43*, 21−30.

https://doi.org/10.1021/acs.jafc.3c08898
J. Agric. Food Chem. 2024, 72, 8423−8433